PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Valerie CHEYNET-SAUVION et al.

Application No.: 09/402,131

Filed: December 8, 1999

BOX: SEQUENCE

Group Art Unit: 1643

Examiner: B. Sisson

Docket No.: 104458

For: RNA-DEPENDENT RNA POLYMERASE FUNCTIONING PREFERABLY ON RNA MATRIX AND PROMOTER-DEPENDENT TRANSCRIPTION PROCESS WITH SAID RNA-DEPENDENT RNA POLYMERASE

SUPPLEMENTAL PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 21, at the end of line 33, insert --(SEQ ID NO: 6)--.

Page 22, line 1, after "(NCGYRRCRASGVLTTSCGNTLTCYI)" insert --(SEQ ID NO: 7)--;

line 2, after "(HNTTLGIPQGSVVSPILCNIFLDKL)" insert --(SEQ ID NO: 8)--;

line 26, after "anti-MRGSHHHHHH" insert --(SEQ ID NO: 9)--; and

line 34, after "MRGSHHHHHHSVLE" insert --(SEQ ID NO: 10)--.

Page 29, at the end of line 27, insert --(SEQ ID NO: 2)--;

line 30, after "3′" (first occurrence) insert --(SEQ ID NO: 11)--; and

line 34, after "3′" insert --(SEQ ID NO: 12)--.

Page 30, line 1, after "5′" insert --(SEQ ID NO: 13)--.

inserted at B1

At the end of the application, please insert the attached paper and computer readable copies of the Sequence Listing.

IN THE CLAIMS:

Please cancel claims 1-34 without prejudice or disclaimer.

Please add the following new claims 35-68:

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--35. A method of amplifying an RNA target sequence, by transcription under the control of a promoter, in an RNA sample comprising said target sequence, said method comprising bringing said sample into contact:

- with a reagent capable of hybridizing with RNA comprising said target sequence,
- in the absence of deoxyribonucleoside triphosphates,
- and with an enzymatic system comprising an RNA-dependent RNA polymerase activity, under conditions allowing the hybridization of said reagent with said RNA comprising said target sequence and under conditions allowing the functioning of said RNA-dependent RNA polymerase activity;

wherein said reagent contains:

(i) a first nucleotide strand comprising: a) a first nucleotide segment capable of playing the role of sense strand of a promoter for said RNA polymerase activity and b) downstream of said first segment, a second nucleotide segment comprising a sequence capable of hybridizing with a region of said RNA, and

(ii) in the hybridized state on the first strand, a second nucleotide strand comprising a third nucleotide segment capable of hybridizing with said first segment so as to form with it a functional double-stranded promoter;

and wherein said RNA polymerase activity is capable of transcribing an RNA template, in the presence of said reagent hybridized with said template, in the absence of associated protein factor and in the absence of a ligase activity.--

--36. A method according to claim 35, wherein said third segment is flanked, at its upstream end, by a fourth nucleotide segment which is shorter than said second segment of the first strand.--

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--37. A method according to claim 36, wherein said fourth segment is capable of hybridizing with a portion opposite said second segment.--

--38. A method according to claim 36, wherein said fourth segment of said second strand is chosen from those whose sequence facilitates the initiation of transcription for said RNA polymerase.--

--39. A method according to claim 36, wherein said second segment of said first strand contains a number of nucleotides at least equal to the sum of the number of nucleotides of said fourth segment, if it is present, and of the number of nucleotides of said sequence of the second segment which is capable of hybridizing with said region of said RNA.--

--40. A method according to claim 35, wherein said first and third segments consist of DNA.--

--41. A method according to claim 35, wherein said fourth segment consists of DNA.--

--42. A method according to claim 35, wherein said RNA polymerase is a virus or phage wild-type RNA polymerase.--

--43. A method according to claim 42, wherein said polymerase is from a family of RNA polymerases selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.--

--44. A method according to claim 42, wherein said RNA polymerase is derived by mutation from an RNA polymerase from a family of RNA polymerases selected from the group consisting of T7, T3 and SP6 RNA polymerases.--

--45. A method according to claim 44, wherein said RNA polymerase contains at least one mutation in the region corresponding to the T7 RNA polymerase sequence containing amino acids 625 to 652.--

--46. A method according to claim 45, wherein said RNA polymerase is capable of transcribing a polynucleotide target sequence with a better yield when said target sequence consists of RNA than when it consists of DNA.--

--47. A method according to claim 35, wherein said enzyme system contains only RNA polymerase activity.--

--48. An RNA polymerase, capable of transcribing, under the control of a promoter, a polynucleotide target of interest of a sequence contained in a polynucleotide template, by synthesizing, in the presence of said template and in the absence of associated protein factor, a product of transcription containing an RNA sequence complementary to said sequence, and said RNA polymerase being capable of synthesizing said product of transcription with a better yield when said target sequence of said template consists of RNA than when it consists of DNA.--

--49. An RNA polymerase according to claim 48, wherein the ratio of the yield of product of transcription of the RNA template to the yield of product of transcription of the DNA template is greater than 2.--

--50. An RNA polymerase according to claim 49, wherein said ratio is greater than 10.--

--51. An RNA polymerase according to claim 48, wherein said RNA polymerase is derived by mutation from a virus or phage RNA polymerase.--

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--52. An RNA polymerase according to claim 51, wherein said phage is an E. coli phage.--

--53. An RNA polymerase according to claim 48, wherein said RNA polymerase possesses a protein sequence homology greater than 50% with a wild-type RNA polymerase of the family of DNA-dependent RNA polymerases selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.--

--54. An RNA polymerase according to claim 53, wherein said RNA polymerase possesses a protein sequence homology greater than 80% with the wild-type RNA polymerase of said family of DNA-dependent RNA polymerases selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.--

--55. An RNA polymerase according to claim 53, wherein said RNA polymerase contains at least one mutation in a region corresponding to the T7 RNA polymerase sequence containing amino acids 625-652.--

--56. An RNA polymerase according to claim 55, wherein said RNA polymerase has the composition of a wild-type DNA-dependent RNA polymerase, except that it contains at least one mutation in said region.--

--57. An RNA polymerase according to claim 55, wherein said RNA polymerase contains at least one mutation at a position corresponding to one of positions 627, 628, 631, 632 and 639 of the T7 RNA polymerase amino acid sequence.--

--58. An RNA polymerase according to claim 55, wherein said mutation comprises the replacement of an amino acid residue, selected from the group consisting of arginine, lysine, serine and tyrosine, of the wild-type RNA polymerase, with another amino acid residue.--

--59. An RNA polymerase according to claim 58, wherein said amino acid replaced is an arginine or a lysine and/or wherein said other amino acid residue is an alanine, valine, leucine, isoleucine, glycine, threonine or serine residue.--

--60. An RNA polymerase according to claim 55, wherein said mutation comprises the replacement of all or part of said region with a homologous region present in a wild-type RNA-dependent polymerase.--

--61. A gene encoding an RNA polymerase as defined in claim 48.--

--62. An expression vector into which a gene as defined in claim 61 is inserted, said vector being capable of expressing said RNA polymerase in a host cell.--

--63. A host cell containing an expression vector as defined in claim 62.--

--64. A method of producing an RNA polymerase as defined in claim 48, said method comprising:

a) obtaining a gene encoding a wild-type RNA polymerase,

b) performing at least one mutation on said gene,

c) inserting the mutated gene obtained into an expression vector,

d) expressing said vector in a host cell in order to obtain a mutated RNA polymerase, and

e) among the mutated RNA polymerases obtained, selecting those which exhibit at least one of the properties of said RNA polymerase to be produced.--

--65. A method of transcription of a template strand comprising an RNA target sequence, said method comprising bringing the template strand into contact with an RNA polymerase capable of transcribing an RNA template, under the control of a promoter, in the absence of auxiliary protein factor, wherein said RNA polymerase is selected from the group consisting of T7 RNA polymerase, SP6 RNA polymerase and the RNA polymerases as defined in claim 48.--

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--66. A method of transcription of a template strand comprising an RNA target sequence, said method comprising bringing the template strand into contact with an RNA polymerase capable of transcribing an RNA template, under the control of a promoter, in the absence of auxiliary protein factor, wherein said template strand consists of: (a) RNA from one of positions +1 to +5 up to the 5' end of the template strand, and (b) DNA from said position up to the 3' end of the template strand when said 3' end does not coincide with said position.--

--67. A method according to claim 66, wherein said RNA polymerase is a virus or phage wild-type RNA polymerase.--

--68. A method according to claim 67, wherein said RNA polymerase is selected from the group consisting of T7, T3 and SP6 RNA polymerase.--

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REMARKS

Claims 35-68 are pending. Claims 1-34 are canceled and claims 35-68 are added herein.

The attached paper and computer readable copies of the Sequence Listing are submitted in compliance with 37 C.F.R. §§1.821-1.825. The contents of the paper copy and the computer readable copy of the Sequence Listing are the same. No new matter is added.

Support for the information provided in the Sequence Listing can be found on pages 21, 22, 29, 30 and 37 of the specification.

Early and favorable consideration on the merits is respectfully requested.

Respectfully submitted,

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Attachments:
Sequence Listing (paper and computer readable copies)

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